In 1953, two researchers, namely James Watson and Francis Crick, discovered the basic structure of DNA. DNA is basically a long molecule that stores coded instructions for the cell. All cells are in some way encoded in the DNA- the DNA provides a basic blueprint that is responsible for the creation and functioning of cells. The information contained in it dictates which cells should grow and when a particular cell should die and how cells should be structured into creating various body parts. For example, the DNA is responsible for determining the quality of our hair, the color and the abundance, or the lack of it. We resemble our parents because our bodies have been formulated by the DNA guiding the process, the DNA that we inherit from them.

DNA and Nucleotides

DNA stands for Deoxyribonucleic acid. It is present in almost all organisms and it stores long term information that is used to construct an organic body.

DNA comprises of a long molecule analogous to a chain, while the links of the chain are called Nucleotides. There are four different nucleotides in the DNA, namely adenine, guanine, cytosine and thymine. They are also called as “A”, “G”, “C” and “T”.

DNA Sequencing

DNA Sequencing entails several techniques and methods that are used to determine the sequence of the aforementioned nucleotide bases in a DNA molecule.

Understanding of DNA sequences has become an integral part of biological research. However, it has been an uphill battle for scientists and researchers to develop and share the core idea of DNA sequencing. But DNA sequencing has come a long way since the 1970s, when the first techniques were introduced.

The Need for DNA Sequencing

The process of DNA sequencing translates the DNA of a specific organism into a format that is decipherable by researchers and scientists. DNA sequencing has given a massive boost to numerous fields such as forensic biology, biotechnology and more. By mapping the basic sequence of nucleotides, DNA sequencing has allowed scientists to better understand genes and their role in the creation of the human body.

Forensic biology uses DNA sequences to identify the organism which it is unique to. Although identifying an individual is less accurate currently, but as the processes evolves further, direct comparisons of large DNA segments, and maybe even genomes, will be more practical and viable and will allow precise identification of an individual. Scientists will be able to isolate the genes responsible for genetic diseases like Cystic Fibrosis, Alzheimer's disease, myotonic dystrophy, etc., which are caused by the inability of genes to work properly.

Agriculture has been helped immensely by DNA sequencing. It has allowed scientists to make plants more resistant to insects and pests, by understanding their genes. Likewise, the same technique has been utilized to increase the productivity and quality of the milk, as well as the meat, produced by livestock.

The DNA Structure

DNA structure resembles to that of a double helix and is composed of three components – alternating sugars, phosphates and one of the four bases.

When a cell divides and the DNA is to be replicated, the double helix is divided, and enzymes called polymerases use each of the two halves as the template for a new opposing strand. Polymerase causes the hydroxyl group at the end to react and link together to form the link of the chain. DNA sequencing relies on the process of DNA duplication.

Sequencing Methods

DNA sequencing attempts to understand the order of bases along the strand. The process of DNA sequencing can be terminated in precise locations and the bases can be isolated where it stops.

1) Maxam-Gilbert Sequencing

The Maxam-Gilbert technique relies on the cleaving of nucleotides by chemical and is most efficient with small nucleotide polymers. This technique was developed by Maxam-Gilbert in 1976-1977 and was published two years after the enlightening papers on Plus-Minus sequencing by Sanger and Coulson. Unlike in Sanger's initial method, which required that each read start be cloned for the synthesis of single-stranded DNA, this method used the purified DNA directly, which made it very popular.

Although due to the advancements in chain termination methodology, the Maxam-Gilbert method has become redundant. It was made obsolete due to it being less ergonomically feasible. It is also considered unsafe because of the extensive use of toxic chemicals.

2) Chain Termination methods

The simplest way to do chain sequencing is to manipulate the chemistry of the molecule. Instead of catering to DNA with normal nucleotides, it's possible to synthesize one in absence of the hydroxyl group, which is essential for the polymerase that adds to the next base. This technique is also known as Sanger method and is named after the discoverer Fredrick Sanger.

The archetypal chain reaction requires a DNA template, DNA polymerase, normal dexoynucleotide (dNTP) and modified deoxynucleotides (ddNTP). The strand synthesis is carried out four times separately, which involves the reaction with ddNTP. This terminates the reaction due to the lack of hydroxyl that is essential for the formation of bond between two nucleotides. The result is four discrete families of polynucleotides.

The polyacrylamide gel electrophoresis is used to denature the DNA to obtain the newly synthesized strands from the given template. High voltage is utilized to heat up the gel to 60

degree centigrade and this makes sure that the two strands don't re-associate. Autoradiography helps in determining the strands as they are radio labeled.

3) Dye-terminators

Dye-terminator sequencing involves labeling of the chain terminating ddNTPs, which allows the sequencing in single reaction, instead of four parallel reactions. In this process, each of the ddNTP is labeled with a fluorescent dyes, which emit light in different wavelengths.

Owing to it is convenience and speed, dye terminator sequencing is the preferred in automated sequencing. Its limitations include anomalies in the incorporation of dye-labeled chain terminators into DNA fragments, which can result in abnormal readings in electronic DNA sequence trace chromatography after the capillary electrophoresis.

The Future

DNA sequencing, in time, may allow us to manipulate the process of evolution. Diseases will vanish, and the human race will be stronger, smarter and better than ever before.